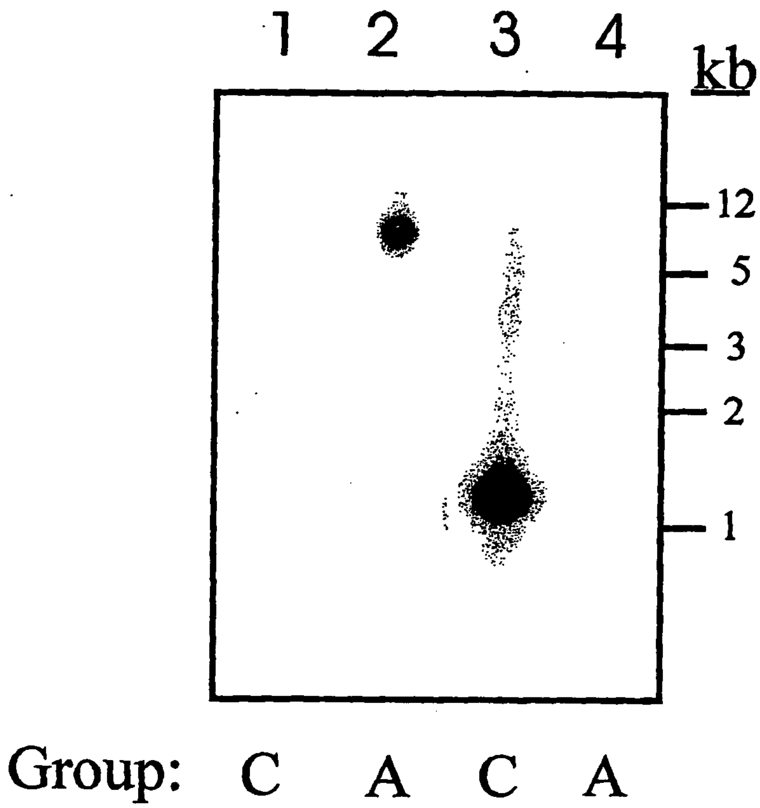


FIG. 1

```
cvHAS  MG--KNIIIM VSWYTIITS- --------NL IAVGGASLIL APAITGYVLH   39
seHAS  MRTLKNLIT- ---------- ---------V VAFSIFWVLL I-----YVNV   25
spHAS  VPIFKKTLI- ---------- ---------V LSFIFLISIL I-----YLNM   25
huHAS  MHCERFLCIL RI---IGTTL ---------- ----FGVSLL LGITAAYIVG   33
xlHAS  MK-EKAAETM EIPEGIPKDL EPKHPTLWRI IYYSFGVVLL ATITAAYVAE   49

cvHAS  WNIALST--I WGVSAYGIFV FGFFLAQVLF SELNRKRLRK WISLRPKGWN   87
seHAS  YLFGAKG--- -SLSIYGFLL IAYLLVKMSL SFF-YKPFKG R---AGQ--Y   65
spHAS  YLFGT-S--- -TVGIYGVIL ITYLVIKLGL SFL-YEPFKG N---PHD--Y   64
huHAS  YQFIQTDNYY FSFGLYGAFL ASHLIIQSLF AFLEHRKMKK SLETPIK--L   81
xlHAS  FQVLKHEAIL FSLGLYGLAM LLHLMMQSLF AFLEIRRVNK S-ELPCS--F   96

cvHAS  DVRLAVIIAG YREDPYMFQK CLESVRDSDY GNVA-RLICV IDGDEDDDMR  136
seHAS  K--VAAIIPS YNEDAESLLE TLKSVQQQTY PLAE--IYVV DDGSADETGI  111
spHAS  K--VAAVIPS YNEDAESLLE TLKSVLAQTY PLSE--IYIV DDGSSNTDAI  110
huHAS  NKTVALCIAA YQEDPDYLRK CLQSVKRLTY PG--IKVVMV IDGNSEDDLY  129
xlHAS  KKTVALTIAG YQENPEYLIK CLESCKYVKY PKDKLKIILV IDGNTEDDAY  146

cvHAS  MAAVYKAIYN DN-------- -----IKKPE --------FV LCESDDKEGE  165
seHAS  KR-------- ---IEDYVRD ---------- TGDLSSNVIV HRSEKNQGKR  140
spHAS  QL-------- ---IEEYVNR ---------- EVDICRNVIV HRSLVNKGKR  139
huHAS  MMDIFSEVMG RDKSATYIWK NNFHE-KGPG ETDES----- -----HKESS  168
xlHAS  MMEMFKDVFH GEDVGTYVWK GNYHTVKKPE ETNKGSCPEV SKPLNEDEGI  196

cvHAS  RIDSDF---S RDICVLQPHR GKRECLYTGE QLAKMDPSVN AVVLIDSDTV  212
seHAS  HA-------- ------QAW- ---------AE E--RSDADV- FLTV-DSDTY  163
spHAS  HA-------- ------QAW- ---------AE E--RSDADV- FLTV-DSDTY  162
huHAS  QHVTQLVLSN KSICIMQKWG GKREVMYTAE R--ALGRSVD YVQVCDSDTM  216
xlHAS  NMVEELVRNK RCVCIMQQWG GKREVMYTAE Q--AIGTSVD YVQVCDSDTK  244

cvHAS  LEKDAILEVV YPLACDPEIQ AVAGECKIWN T-DTLLSLLV AWRYYSAFCV  261
seHAS  IYPDALEELL KTFNDPTVFA ATG-HLNVRN RQTNLLTRLT DIRYDNAFGV  212
spHAS  IYPNALEELL KSFNDETVYA ATG-HLNARN RQTNLLTRLT DIRYDNAFGV  211
huHAS  LDPASSVEMV KVLEEDPMVG GVGGDVQILN KYDSWISFLS SVRYWMAFNI  266
xlHAS  LDELATVEMV KVLESNDMYG AVGGDVRILN PYDSFISFMS SLRYWMAFNV  294

cvHAS  ERSAQSFFRT VQCVGGPLGA YKIDIIKEIK DPWISQRFLG QKCTYGDDRR  311
seHAS  ERAAQSVTGN ILVCSGPLSV YRREVVVPNI DRYINQTFLG IPVSIGDDRC  262
spHAS  ERAAQSLTGN ILVCSGPLSI YRREVIIPNL ERYKNQTFLG LPVSIGDDRC  261
huHAS  ERACQSYFGC VQCISGPLGM YRNSLLHEFV EDWYNQEFMG NQCSFGDDRH  316
xlHAS  ERACQSYFDC VSCISGPLGM YRNNILQVFL EAWYRQKFLG TYCTLGDDRH  344

cvHAS  LTNEILMRGK KVVFTPFAVG WSDSPTNVFR YIVQQTRWSK SWCREIWYTL  361
seHAS  LTNYATDLG- KTVYQSTAKC ITDVPDKMST YLKQQNRWNK SFFRESIISV  311
spHAS  LTNYAIDLG- RTVYQSTARC DTDVPFQLKS YLKQQNRWNK SFFRESIISV  310
huHAS  LTNRVLSLGY ATKYTARSKC LTETPIEYLR WLNQQTRWSK SYFREWLYNA  366
xlHAS  LTNRVLSMGY RTKYTHKSRA FSETPSLYLR WLNQQTRWTK SYFREWLYNA  394

cvHAS  FAAWKHGLSG IWLAFECLYQ ITYFFLVIYL FSRLAVEADP RAQTATVIVS  411
seHAS  KKIMNNPFVA LWTILEVSMF MMLVYSVVDF FVGNVREFDW LRVLAFLVII  361
spHAS  KKILSNPIVA LWTIFEVVMF MMLIVAIGNL LFNQAIQLDL IKLFAFLSII  360
huHAS  MWFHKHH--- LWMTYEAIIT GFFPFFLIAT VIQLFYRGKI WNILLFLLTV  413
xlHAS  QWWHKHH--- IWMTYESVVS FIFPFFITAT VIRLIYAGTI WNVVWLLLCI  441

cvHAS  TTVALIKCGY FSFRAKDIRA FYFV-LYTFV YFFCMIPARI TAMMTLWDIG  460
seHAS  FIVALCRNIH YM--LKHPLS FLLSPFYGVL HLFVLQPLKL YSLFTIRNAD  409
spHAS  FIVALCRNVH YM--VKHPAS FLLSPLYGIL HLFVLQPLKL YSLCTIKNTE  408
huHAS  QLVGLIKSS- FASCLRGNIV MVFMSLYSVL YMSSLLPAKM FAIATINKAG  462
xlHAS  QIMSLFKSI- YACWLRGNFI MLLMSLYSML YMTGLLPSKY FALLTLNKTG  490

cvHAS  WDTRGGNEKP SVGTRVALWA KQYLIAYMWW AAVVGAGVYS IVHNWMFDWN  510
seHAS  WGT----RKK L--------- ---------- ---------- -L*         417
spHAS  WGT----RKK V--------- ---------- ---------T IFK*        419
huHAS  WGTSG--RKT IVVNFIGL-- ---IPVSVWF TILLGGVIFT IYKESKRPFS  505
xlHAS  WGTSG--RKK IVGNYMPI-- ---LPLSIWA AVLCGGVGYS IYMDCQNDWS  533

cvHAS  S-----LSYR FALVGIC-SY IVFIVIVLVV YFTGKITTWN FTKLQKELIE  554
huHAS  ES-KQTVLIV GTLLYAC--- --YWVMLLTL YV---VLINK CGRRKKGQQY  546
xlHAS  TPEKQKEMY- -HLLYGCVGY VMYWVIMAVM YW---VWVKR CCR-KRSQTV  577

cvHAS  DRVLYDATTN AQSV*                                        568
huHAS  DMVL----DV*                                             552
xlHAS  TLVH----DI PDMCV*                                       588
```

FIG. 2

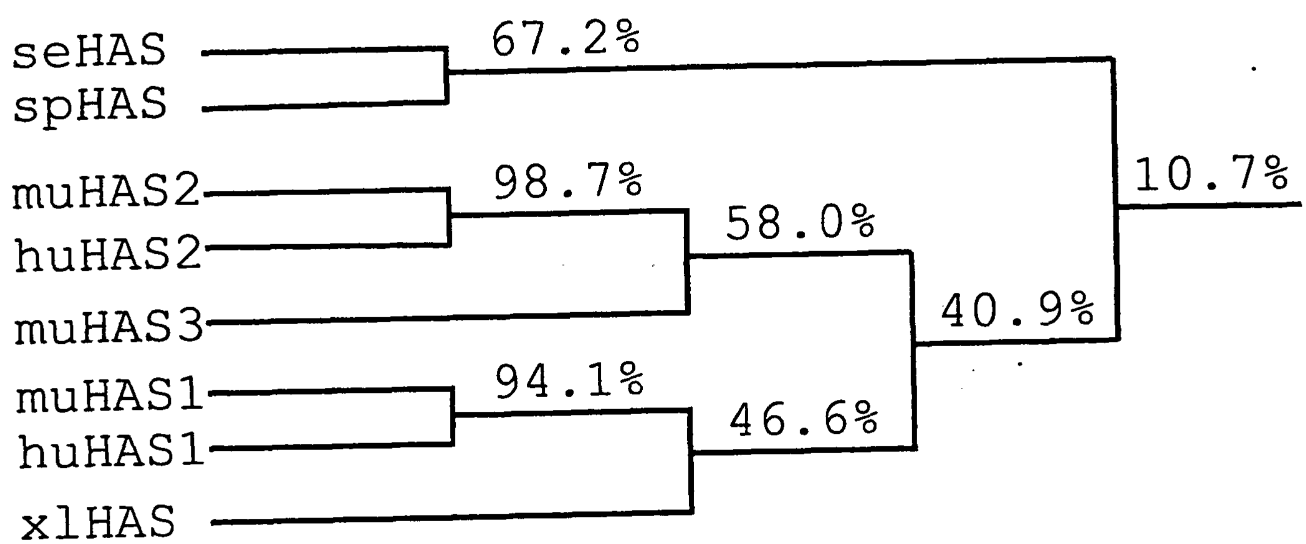

seHAS
spHAS
muHAS2
huHAS2
muHAS3
muHAS1
huHAS1
xlHAS
67.2%
98.7%
58.0%
94.1%
46.6%
40.9%
10.7%

FIG. 3

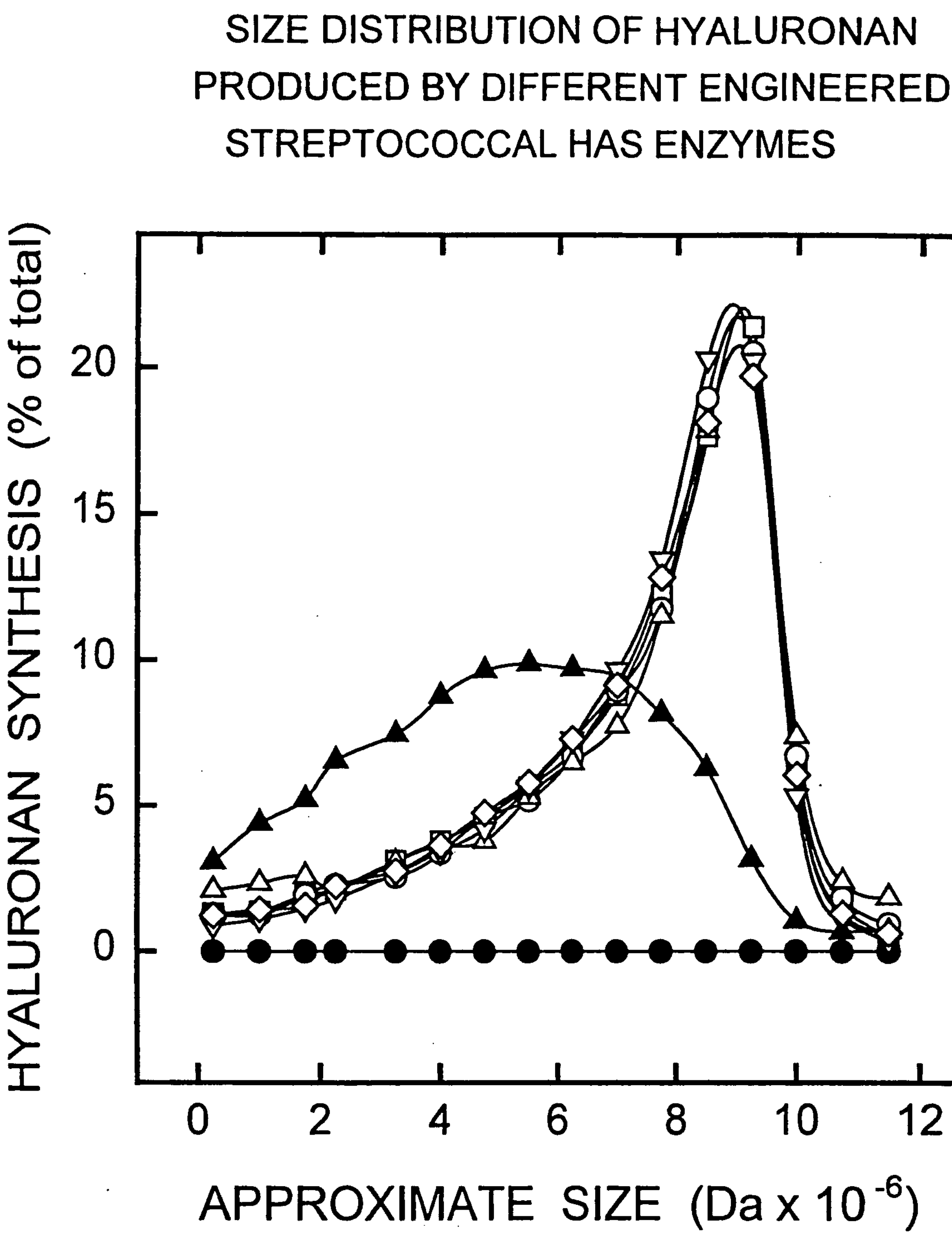

SIZE DISTRIBUTION OF HYALURONAN
PRODUCED BY DIFFERENT ENGINEERED
STREPTOCOCCAL HAS ENZYMES
HYALURONAN SYNTHESIS (% of total)
20
15
10
5
0
0
2
4
6
8
10
12
APPROXIMATE SIZE (Da x 10 -6)

FIG. 4

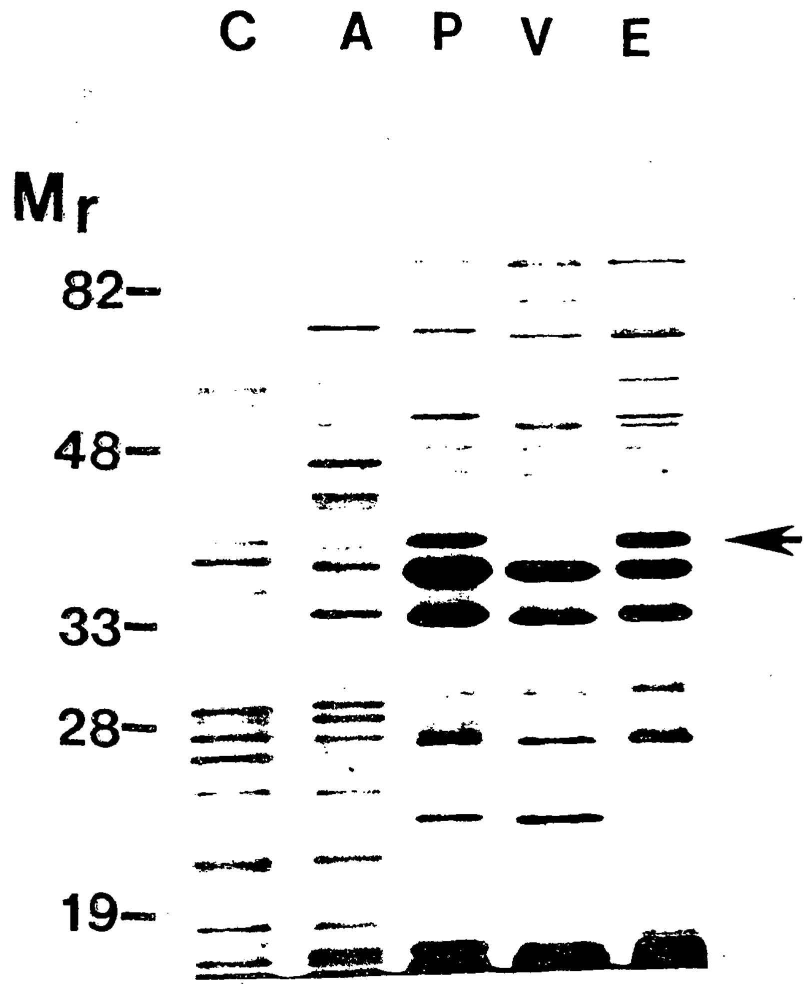


FIG. 5

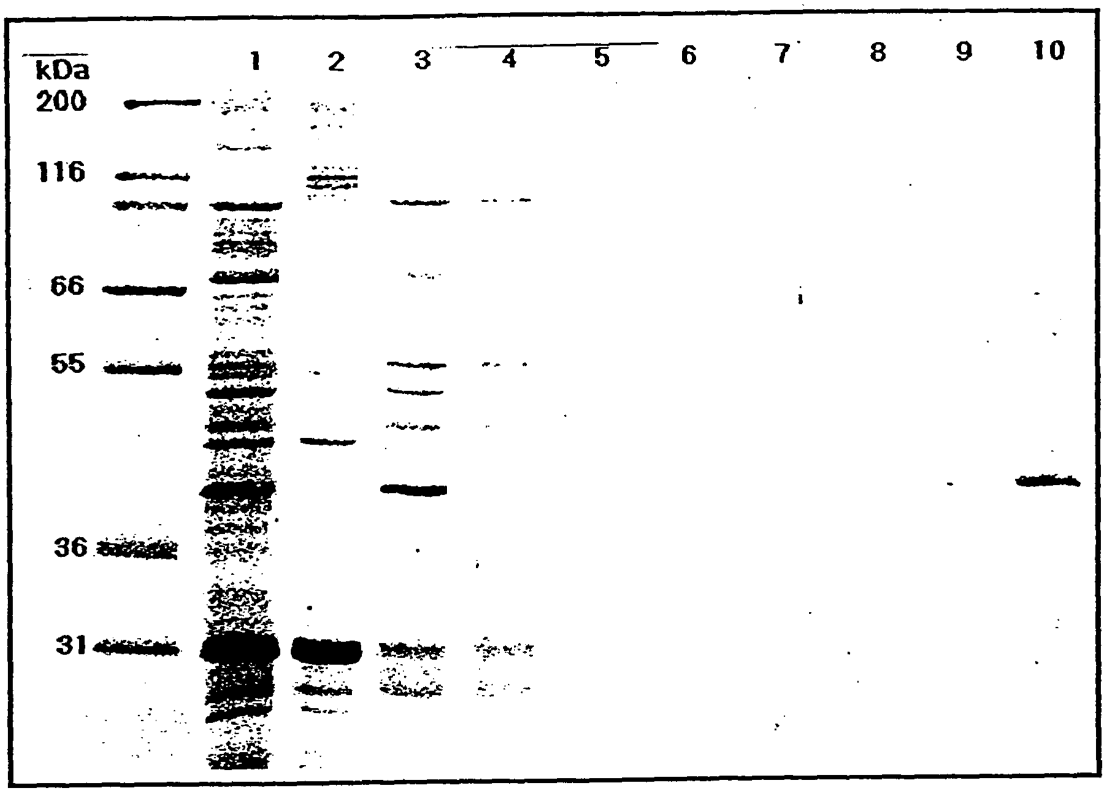


FIG. 6

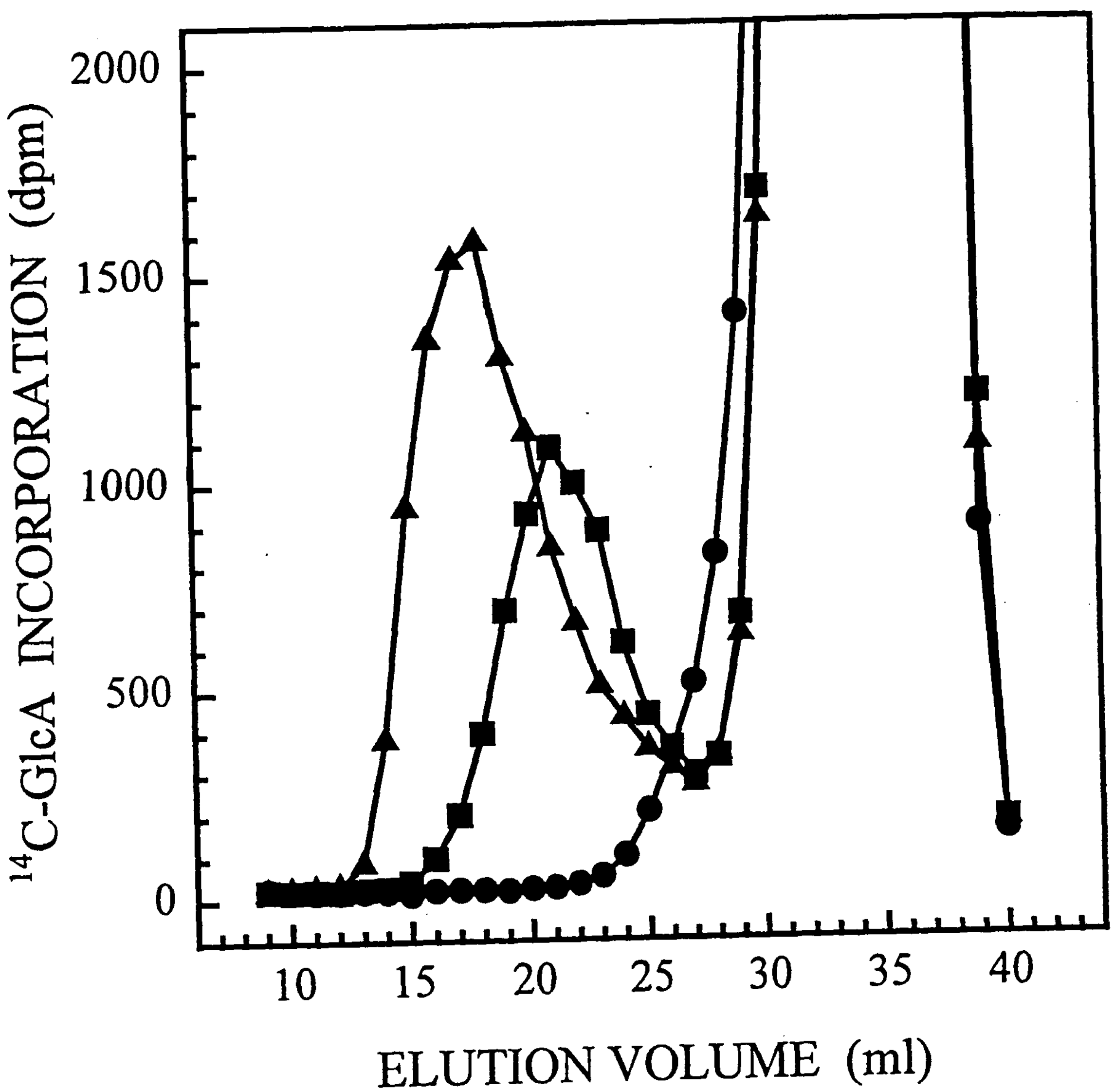

2000
1500
1000
500
0
^{14}C-GlcA INCORPORATION (dpm)
10
15
20
25
30
35
40
ELUTION VOLUME (ml)

FIG. 7

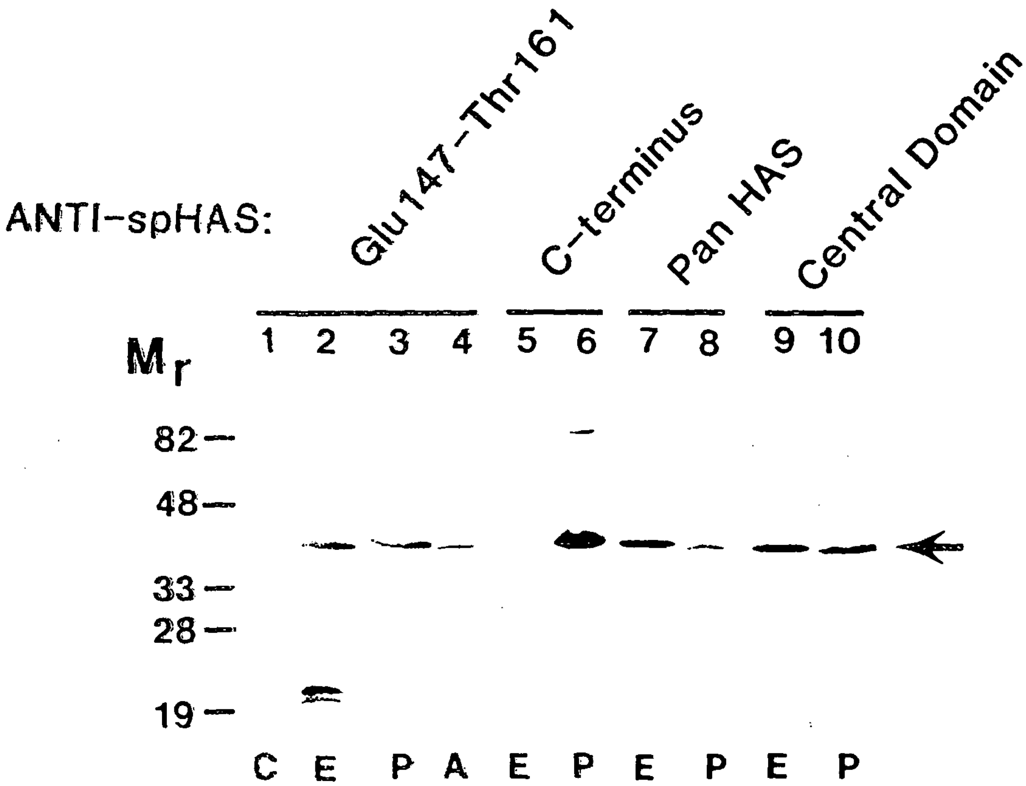
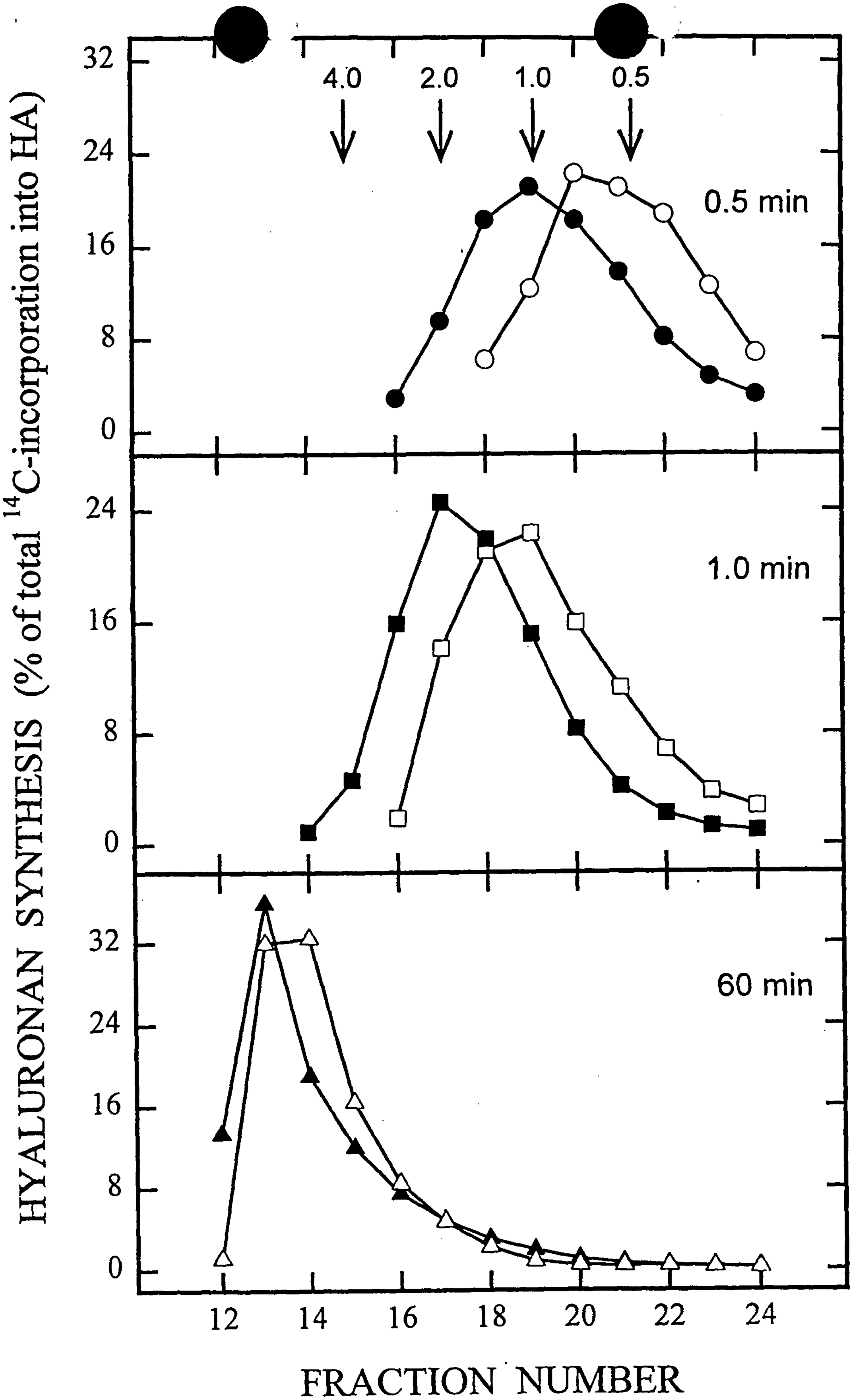

ANTI-spHAS:
Glu147-Thr161
C-terminus
Pan HAS
Central Domain
M_r
1 2 3 4 5 6 7 8 9 10
82
48
33
28
19
C E P A E P E P E P

FIG. 8



HYALURONAN SYNTHESIS (% of total ^{14}C-incorporation into HA)
4.0
2.0
1.0
0.5
0.5 min
1.0 min
60 min
32
24
16
8
0
12
14
16
18
20
22
24
FRACTION NUMBER

FIG. 9

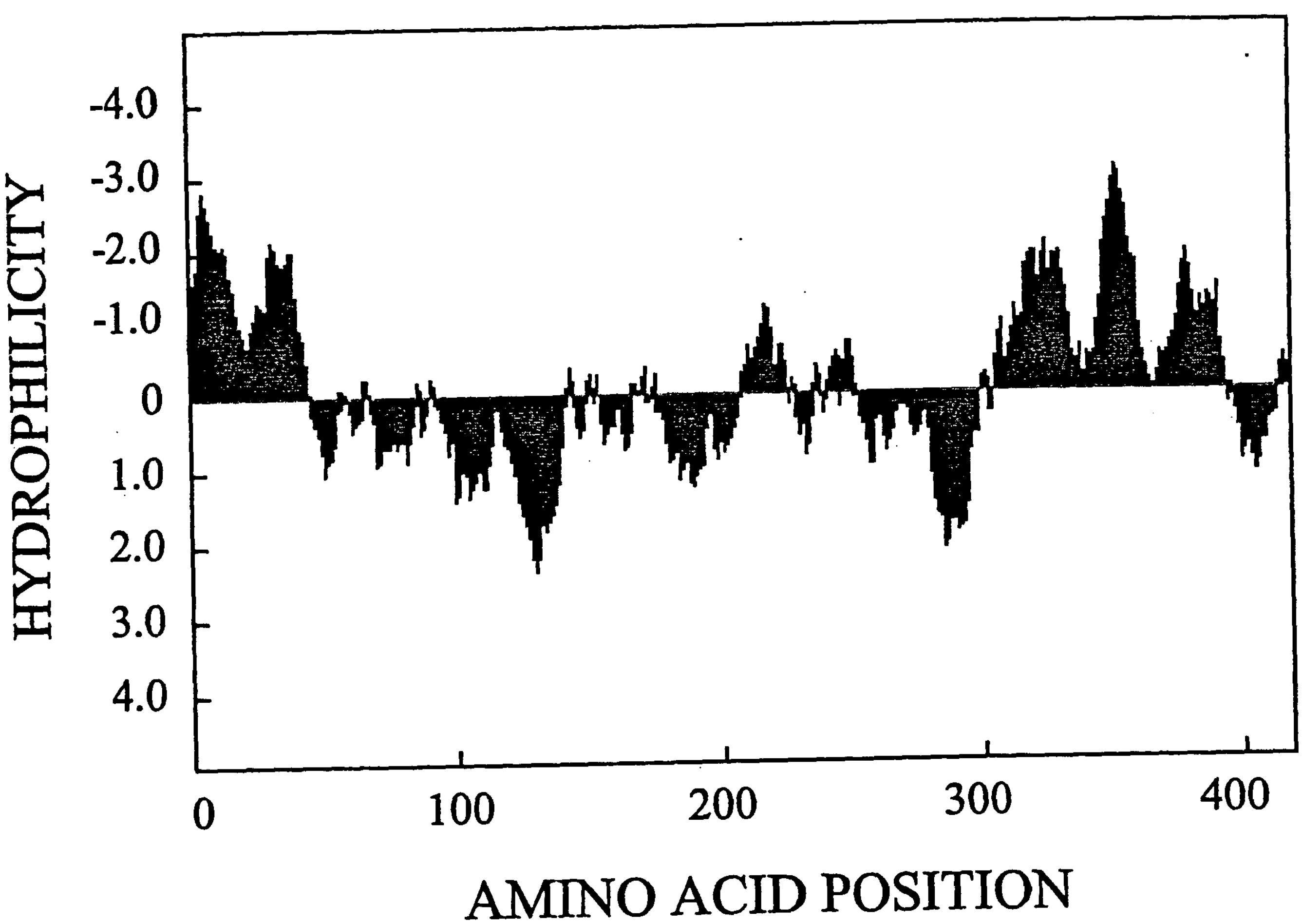

HYDROPHILICITY
-4.0
-3.0
-2.0
-1.0
0
1.0
2.0
3.0
4.0
0
100
200
300
400
AMINO ACID POSITION

FIG. 10

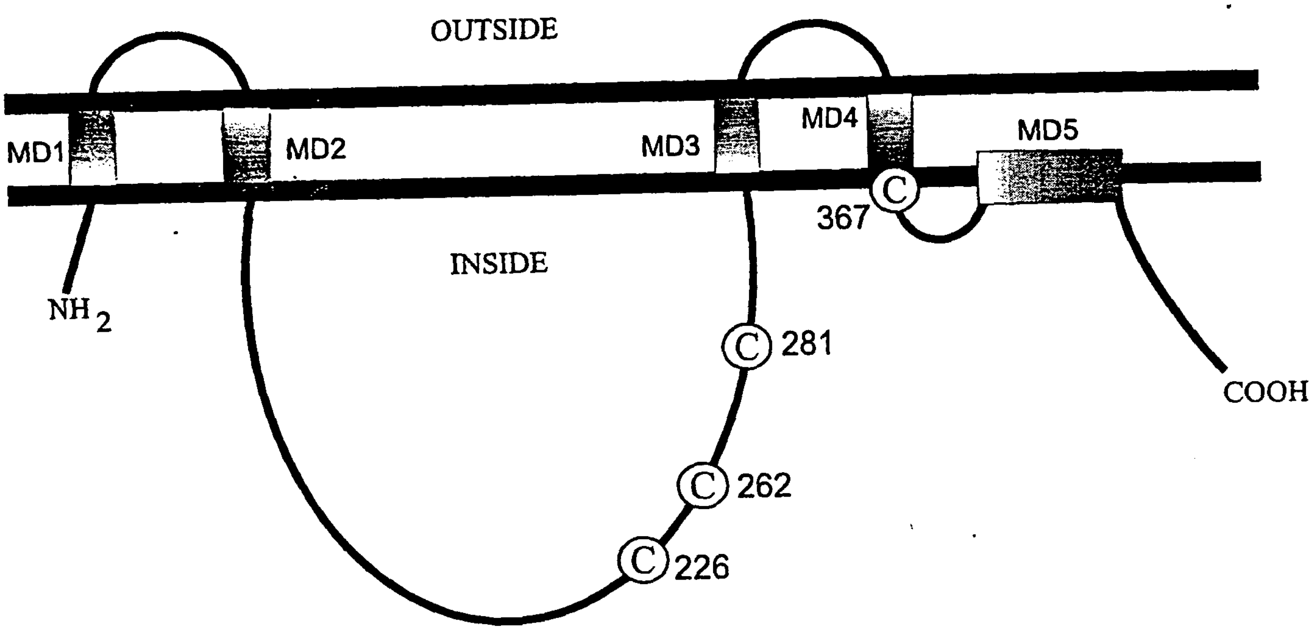

OUTSIDE
MD1
MD2
MD3
MD4
MD5
C
367
INSIDE
NH 2
C 281
C 262
C 226
COOH

FIG. 11

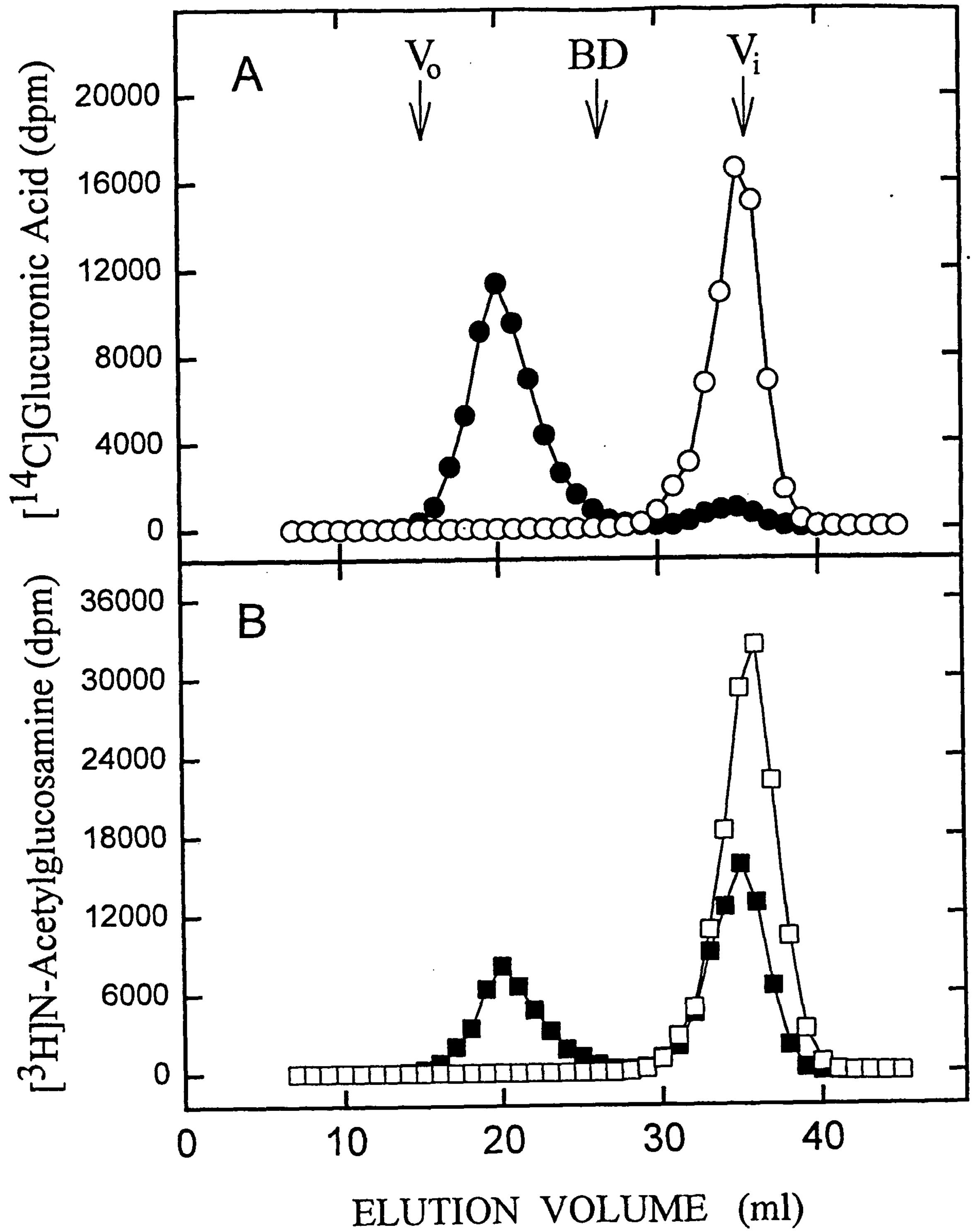

A
B
V_o
BD
V_i
[^{14}C]Glucuronic Acid (dpm)
20000
16000
12000
8000
4000
0
[^{3}H]N-Acetylglucosamine (dpm)
36000
30000
24000
18000
12000
6000
0
0
10
20
30
40
ELUTION VOLUME (ml)

FIG. 12

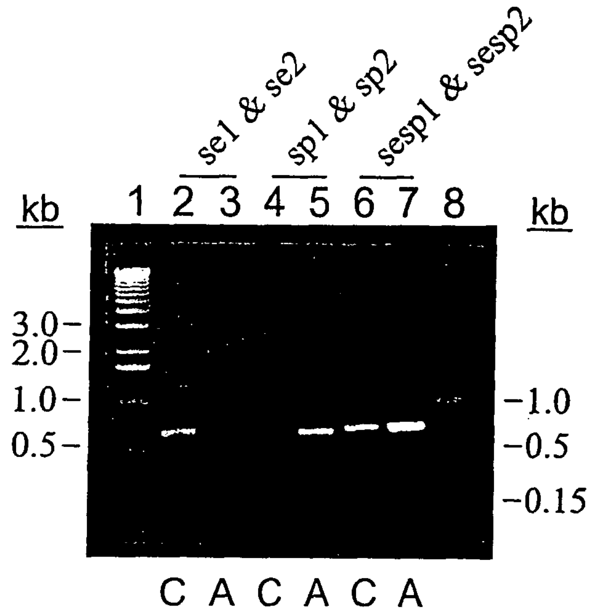

se1 & se2
sp1 & sp2
sesp1 & sesp2
kb
1 2 3 4 5 6 7 8
kb
3.0–
2.0–
1.0–
0.5–
–1.0
–0.5
–0.15
C A C A C A

FIG. 13